

In re Application of

Schelberger et al

Serial No. 09/581,833

Filed: December 15, 1998 as PCT international application

For: FUNGICIDAL MIXTURES BASED ON AMIDE COMPOUNDS AND PYRIDINE  
DERIVATIVES



## DECLARATION

I, Eberhard Ammermann, a doctor of natural sciences, a citizen of the Federal Republic of Germany and residing at 2, von-Gagern-Straße, 64646 Heppenheim, Germany, declare as follows:

I am a fully trained chemist, having studied chemistry at the University of Bonn, Germany, from 1965 to 1974;

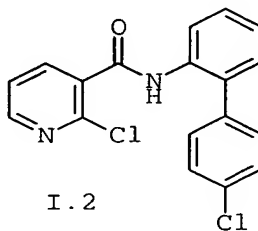
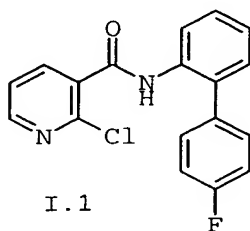
I was awarded my doctor's degree by the said University in 1974; I worked as an assistant at the said University from 1971 to 1975;

I joined BASF Aktiengesellschaft of 67056 Ludwigshafen, Germany, in 1975, and have since then been working in the field of fungicides, synthesizing fungicides and testing substances for their fungicidal activity, and am therefore fully conversant with the fungicidal art;

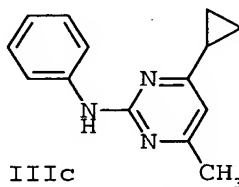
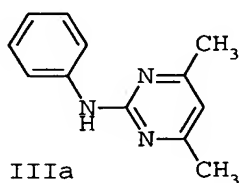
I am one of the inventors of the invention disclosed and claimed in Application Serial No. 09/581,833 and therefore I am familiar with the field to which it belongs. In order to provide further support for the synergistic effect of the mixtures disclosed and claimed in Application Ser. No. 09/581,833 I have conceived the tests described below, which were carried out under my supervision.

The following compounds and compositions containing them were tested:

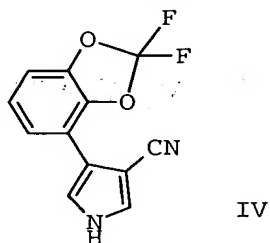
### 1. Amides I.1, I.2



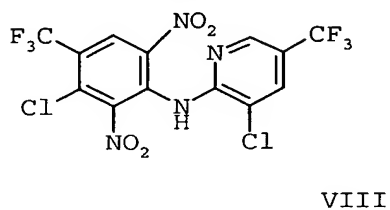
2. Pyrimidine derivatives IIIa, IIIc



3. Pyrrole derivate IV



4. Dinitroaniline VIII



The biological activity of the compounds and compositions containing them were tested as follows:

**Trial report 1:** Control of gray mould (*Botrytis cinerea*) on fruit slices of green pepper

Fruit slices of green pepper were sprayed to run-off with an aqueous suspension, containing the concentration of active ingredient or their mixture mentioned in the table below, prepared from a stock solution containing 10 % of the active ingredient, 85 % cyclohexanone and 5 % emulsifier. After 2 hours the sprayed-on layer had dried, the disks were inoculated with a spore suspension of *Botrytis cinerea* containing  $1.7 \times 10^6$  spores per ml in 2 wt. % aqueous biomalt solution. The infected fruit slices were then incubated in chambers with high humidity for four days at 18-20° C. The fruit slice area under fungal attack was then assessed visually in percent.

These figures were then converted into degrees of control. The degree of control in the untreated fruit slices was set at 0. The degree of control when 0% of the fruit slice area was attacked was set at 100.

The degree of control (W) was calculated in accordance with the Abbott formula as follows:

Abbott formula:  $W = (1 - \alpha/\beta) \cdot 100$

$\alpha$  fungus attack of treated fruit slices [%] and

$\beta$  fungus attack of untreated control fruit slices [%]

The expected degrees of action of the active ingredient compositions were determined in accordance with the Colby formula (Colby, S. R. "Calculating synergistic and antagonistic responses of herbicide Combinations", Weeds, 15, p. 20 - 22, 1967) and compared with the degrees of action observed.

The values for the fungicidal action varied between the individual experiments because the plant material in the individual experiments exhibited varying degrees of attack; for this reason, only the results within the same experiment can be compared with each other.

Colby formula:  $E = x + y - (x \cdot y : 100)$

E = Expected degree of action, expressed in % of the untreated control, when active ingredients A and B are applied together, the concentration of A being [a] and the concentration of B being [b]

x = degree of action of ingredient A, expressed in % of the untreated control, when concentration [a] of the active ingredient A is applied

y = degree of action of ingredient B, expressed in % of the untreated control, when concentration [b] of the active ingredient B is applied

As a general rule the comparison of the expected degree of action ("E" according to the Colby formula) with the degree of action found shows whether the effect is synergistic or not, the correlation being as follows:

degree of action found > "E"  $\Rightarrow$  synergism  
 degree of action found < "E"  $\Rightarrow$  no synergism

The test results are listed in the following tables:

Compound	Appln. Rate [ppm]	degree of control [%]
control (untreated)	(92 % attack)	0
I.1	12,5	35
	6,3	24
	3.1	2
I.2	12.5	67
	6.3	57
	3.1	2
III.a	31	35
III.c	63	35
	31	35
IV	3.1	13
	1.6	2
VIII	6.3	2
	3.1	2

The same experiments were carried out with mixtures in accordance with the instant invention. The results achieved with these compositions are listed in the following table:

Mixture according to the invention Application rate [ppm]	Degree of action observed	Degree of action calculated (Colby)
I.1 + III.a 3.1 + 31 ppm ratio 1 : 10	57	36
I.1 + III.a 6.3 + 31 ppm ratio 1 : 5	89	51
I.1 + III.c 3.1 + 31 ppm ratio 1 : 10	95	36
I.1 + III.c 6.3 + 63 ppm ratio 1 : 10	95	51

Mixture according to the invention Application rate [ppm]	Degree of action observed	Degree of action calculated (Colby)
I.1 + III.c 6.3 + 31 ppm ratio 1 : 5	78	51
I.1 + III.c 12.5 + 63 ppm ratio 1 : 5	89	58
I.1 + IV 3.1 + 1.6 ppm ratio 2 : 1	35	5
I.1 + IV 6.3 + 3.1 ppm ratio 2 : 1	67	34
I.1 + IV 6.3 + 1.6 ppm ratio 4 : 1	46	26
I.1 + IV 12.5 + 3.1 ppm ratio 4 : 1	67	43
I.1 + VIII 6.3 + 3.1 ppm ratio 2 : 1	45	26
I.1 + VIII 12.5 + 6.3 ppm ratio 2 : 1	89	36
I.1 + VIII 12.5 + 3.1 ppm ratio 4 : 1	78	36
I.2 + III.a 3.1 + 31 ppm ratio 1 : 10	100	36
I.2 + III.a 6.3 + 31 ppm ratio 1 : 5	100	72
I.2 + III.c 3.1 + 31 ppm ratio 1 : 10	84	36
I.2 + III.c 6.3 + 63 ppm ratio 1 : 10	99	72
I.2 + III.c 6.3 + 31 ppm ratio 1 : 05	99	72

Mixture according to the invention Application rate [ppm]	Degree of action observed	Degree of action calculated (Colby)
I.2 + III.c 12.5 + 63 ppm ratio 1 : 5	100	79
I.2 + IV 3.1 + 1.6 ppm ratio 2 : 1	67	5
I.2 + IV 6.3 + 3.1 ppm ratio 2 : 1	95	62
I.2 + IV 6.3 + 1.6 ppm ratio 4 : 1	88	72
I.2 + VIII 6.3 + 3.1 ppm ratio 2 : 1	77	58
I.2 + VIII 12.5 + 6.3 ppm ratio 2 : 1	88	68
I.2 + VIII 12.5 + 3.1 ppm ratio 4 : 1	95	68

**Trial report 2:** Protective control of *Botrytis cinerea* on leaves of green pepper

Young seedlings of green pepper of the variety "Neusiedler Ideal Elite" were grown in pots to the 4 to 5 leaf stage. These plants were sprayed to run-off with an aqueous suspension, containing the concentration of active ingredient or their mixture mentioned in the table below, prepared from a stock solution containing 10 % of the active ingredient, 85 % cyclohexanone and 5 % emulsifier. The next day the treated plants were inoculated with a spore suspension of *Botrytis cinerea*, containing  $1.7 \times 10^6$  spores/ml in a 2 % aqueous biomalt solution. Then the trial plants were immediately transferred to a humid chamber. After 5 days at 22 to 24°C and a relative humidity close to 100 % the extent of fungal attack on the leaves was visually assessed as % diseased leaf area.

The test results are listed in the following tables:

Compound	Appln. Rate [ppm]	degree of control [%]
control (untreated)	(85 % attack)	0
I.1	12,5	0
	6,3	0
	3.1	0
I.2	6.3	42
	3.1	13
III.a	63	71
	31	71
III.c	63	42
	31	13
IV	3.1	71
	1.6	42
VIII	3.1	71
	1.6	42

The same experiments were carried out with mixtures in accordance with the instant invention. The results achieved with these compositions are listed in the following table:

Mixture according to the invention Application rate [ppm]	Degree of action observed	Degree of action calculated (Colby)
I.1 + III.a 3.1 + 31 ppm ratio 1 : 10	91	71
I.1 + III.a 6.3 + 63 ppm ratio 1 : 10	100	71
I.1 + III.c 3.1 + 31 ppm ratio 1 : 10	100	13
I.1 + III.c 6.3 + 63 ppm ratio 1 : 10	100	42
I.1 + III.c 6.3 + 31 ppm ratio 1 : 5	100	13
I.1 + III.c 12.5 + 63 ppm ratio 1 : 5	100	42
I.1 + IV 3.1 + 1.6 ppm ratio 2 : 1	85	42
I.1 + IV 6.3 + 3.1 ppm ratio 2 : 1	100	71

<b>Mixture according to the invention</b> <b>Application rate [ppm]</b>	<b>Degree of action observed</b>	<b>Degree of action calculated (Colby)</b>
<b>I.1 + IV</b> 6.3 + 1.6 ppm ratio 4 : 1	100	42
<b>I.1 + IV</b> 12.5 + 3.1 ppm ratio 4 : 1	100	71
<b>I.1 + VIII</b> 3.1 + 1.6 ppm ratio 2 : 1	100	42
<b>I.1 + VIII</b> 6.3 + 1.6 ppm ratio 4 : 1	100	42
<b>I.1 + VIII</b> 12.5 + 3.1 ppm ratio 4 : 1	97	71
<b>I.2 + III.a</b> 3.1 + 31 ppm ratio 1 : 10	100	74
<b>I.2 + III.a</b> 6.3 + 63 ppm ratio 1 : 10	100	83
<b>I.2 + III.a</b> 6.3 + 31 ppm ratio 1 : 5	100	83
<b>I.2 + III.c</b> 3.1 + 31 ppm ratio 1 : 10	97	24
<b>I.2 + III.c</b> 6.3 + 63 ppm ratio 1 : 10	97	66
<b>I.2 + III.c</b> 6.3 + 31 ppm ratio 1 : 05	100	50
<b>I.2 + IV</b> 3.1 + 1.6 ppm ratio 2 : 1	100	50
<b>I.2 + IV</b> 6.3 + 1.6 ppm ratio 4 : 1	91	66
<b>I.2 + VIII</b> 3.1 + 1.6 ppm ratio 2 : 1	100	50
<b>I.2 + VIII</b> 6.3 + 3.1 ppm ratio 2 : 1	100	83



Mixture according to the invention Application rate [ppm]	Degree of action observed	Degree of action calculated (Colby)
I.2 + VIII 6.3 + 1.6 ppm ratio 4 : 1	100	66

These test results clearly demonstrate that compositions comprising compounds I.1, I.2, IIIa, IIIc, IV, and VIII exhibit synergism at different application rates.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at 67056 Ludwigshafen, Germany, this <sup>23<sup>rd</sup></sup> day of November, 2001.

Eberhard Surmann

Signature of Declarant